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PLACENTAL TRANSFER OF PERCHLORATE AND TRIIODOTHYRONINE IN THE GUINEA PIG

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THIS report documents (1) production of fetal goiter when perchlorate is administered to pregnant guinea pigs, (2) failure of large doses of triiodothyronine to protect the fetus against perchlorate induced goiter, and (3) studies on the placental transfer of I^{131} labeled triiodothyronine. The findings bear upon the use of these agents in the treatment of thyroid diseases during pregnancy. In addition, they may be relevant to broader aspects of fetal-maternal interrelations.

METHODS

Administration of $KClO_4$ and Triiodothyronine to Pregnant Guinea Pigs

Sixteen guinea pigs in the second or third week of pregnancy were treated as follows. One control group of three pigs received a diet[1] containing 0.48 μg. iodine per gram. distilled water *ad libitum*, and 50 mg. of ascorbic acid orally twice a week. The other pigs were offered a 1% solution of $KClO_4$ instead of distilled water. They were divided into 4 groups which received 0, 8, 16, and 32 μg., respectively, of *l*-3-5-3′-triiodothyronine[2] by subcutaneous injection each day. The solution of triiodothyronine (T-3) was prepared every three days in .05 ml. of 0.1 N NaOH and diluted with 0.9% NaCl. Control animals and those receiving $KClO_4$ alone were given daily injections of saline solution. Initial *ad libitum* consumption of the 1% $KClO_4$ solution was the same as the intake of distilled water in the control group, but after the first week perchlorate intake decreased progressively, particularly in animals receiving no T-3. The over-all mean dose of $KClO_4$ was 152 mg./100 gm. body weight per 24 hours in T-3 injected pigs and 74 mg./100 gm. in the group receiving only $KClO_4$. The mean duration of treatment was 37 days (range 21–48).

One hour after 10–20 microcuries of carrier free NaI^{131} was administered subcutaneously to the mother, the near-term fetuses were removed by hysterotomy under pentobarbital anesthesia. Blood was obtained from the hearts of mothers and fetuses for determination of I^{131} content in a well-type scintillation detector. Fetal and maternal thyroids were excised, weighed, fixed in 5% formaldehyde, and assayed for radioactivity. The counts per minute per gram of thyroid tissue, divided by the counts per minute per ml. of plasma defines the "T/S" ratio.[3]

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[1] Purina rabbit chow.

[2] Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

[3] "T/S" ratio as defined here differs from the conventional thyroid/plasma ratio for inorganic iodine. Expressing the thyroidal uptake of I^{131} in this way takes into consideration non-uniform distribution of inorganic I^{131} between adult and fetus (1).

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Administration of $KClO_4$ to Non-Pregnant Adult Female Guinea Pigs

To determine whether or not goiter could be produced in non-pregnant pigs under the conditions of the experiment described above, 24 young adult virgin sows were divided into two groups, one receiving distilled water, the other 1% $KClO_4$ *ad libitum*. Four controls and four $KClO_4$ treated animals were sacrificed at 30, 60, and 90 days after the beginning of the experiment. "T/S" ratios were determined as described above. In addition, one-half of each gland was analyzed for total iodine content by the method of Barker (1).

Placental Transfer of I^{131} labeled l-3-5-3'-Triiodothyronine

Eighteen studies of the partition of I^{131} labeled l-3-5-3'-triiodothyronine (T-3*) in the maternal and fetal circulations were done. T-3*, prepared by radioiodination of 3-5-diiodothyronine, purified by column chromatography and dissolved in 50% propylene glycol[4] was administered to near term sows one-half hour after an oral dose of 330 mg. of $KClO_4$. At intervals from 5 minutes to 24 hours after the T-3* injection, fetuses were delivered as described above. Appropriate changes of rubber gloves and surgical instruments and use of separate working areas maintained isolation of the fetal and maternal circulations. Fetal and maternal blood samples obtained simultaneously were heparinized and processed as described below. Radioactivity in the thyroid glands of mothers and fetuses was measured to show complete inhibition of iodide trapping. T-3* was injected into a jugular vein exposed under light ether anesthesia in the experiments in which the pigs were sacrificed at one hour or earlier. In all other experiments, i.e., at 4, 12, and 24 hours, it was given subcutaneously. In experiments lasting longer than 4 hours, oral doses of 166 mg. of $KClO_4$ were administered every 6 hours in addition to the initial dose given one-half hour before the T-3* injection. T-3 dosage varied with the specific activity of various lots (15 to 78 millicuries per milligram) and the counting rate demands of the particular experiment. The smallest T-3 dose was 0.3 μg., the largest, 10 μg.

Blood was centrifuged immediately and a small crystal of NaS_2O_3 added to the separated plasma which was acidified to pH 2, and extracted three times with four volumes of thiosulfate saturated n-butyl alcohol. The pooled supernatant was neutralized with NH_4OH and evaporated to dryness. The residue was taken up in 0.1–0.2 ml. of a solution of 3 parts of ethyl alcohol and one of NH_4OH immediately prior to application to Whatman #3 filter paper. Chromatograms were developed in ascending fashion in butanol-dioxane-ammonia and butanol-acetic acid-water. The sites of previously added carriers were identified by coupling with the diazonium salt of sulfanilic acid. When counting rates were favorable, the distribution of radioactivity on the chromatogram was determined using an automatically driven scanning device with an end window detector, a counting rate meter and recorder. Otherwise, as in the case of fetal plasmas, segments of chromatograms were counted in a deep well scintillation counter with a sensitivity of 400,000 counts per microcurie and a background of 15–25 counts per minute. Results are expressed in per cent of administered dose per ml. plasma, corrected to a 1000 gm. body weight. In experiments on the decay of T-3* in newborn guinea pigs, the same analytic techniques were employed. In one experiment, whole fetal carcasses were homogenized. Acidified aliquots were extracted with butanol and chromatographed in the same way as noted above for plasma.

RESULTS

A. *The Effects of Perchlorate with and without Simultaneous Administration of Triiodothyronine*

Feeding 1% perchlorate to pregnant guinea pigs during the last 21 to 48 days of gestation resulted in massive enlargement of the fetal thyroid

[4] Abbott Laboratories, Oak Ridge, Tennessee.

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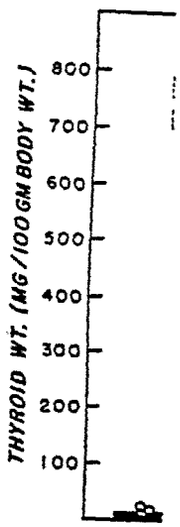

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glands. Their over-all mean weight was 491 ± 157.4 mg./100 gm. body weight, compared with control fetal thyroid weights of 32.3 ± 3.44 mg./100 gm. As shown in Figure 1, large goiters developed despite simultaneous administration of triiodothyronine (T-3) in doses up to 32 μg. per day to the mother. The striking perchlorate effect on the fetus is in marked contrast to the unaltered weights of maternal thyroids. This contrast is exemplified in the illustration (Fig. 2) of the *in situ* appearance of typical highly vascular goiters in both fetuses of a one kilogram mother whose excised thyroid gland is included for reference. In this particular case, the

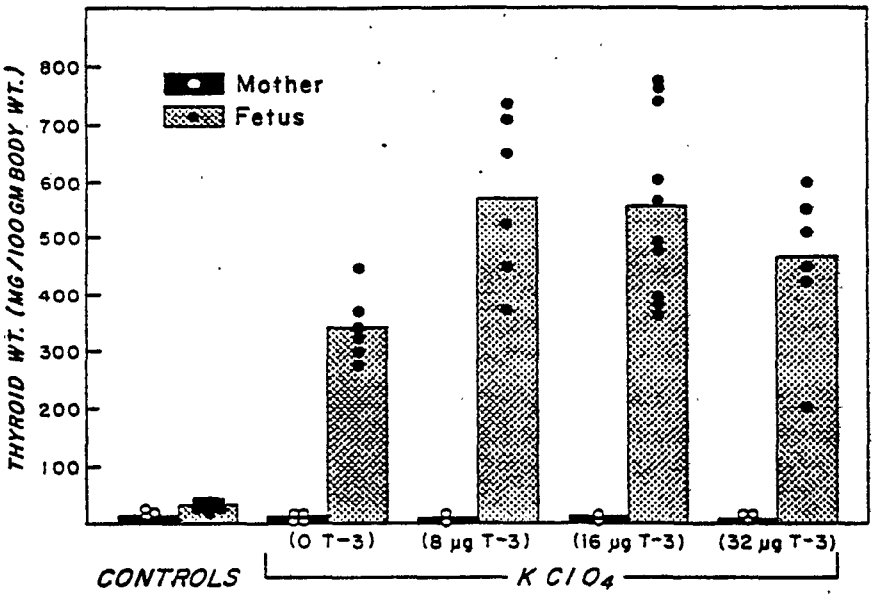


FIG. 1. Thyroid weights (mg./100 gm. body weight) of mothers (black bars) and fetuses (cross-hatched) with and without simultaneous administration of graded doses of triiodothyronine.

sow received 16 μg. T-3 daily. Similarly, microscopic examination showed no changes in maternal glands despite virtual replacement of the normal fetal thyroid architecture by solid cords of cells (Fig. 3). Here, as for the thyroid weights (Fig. 1), it appears that, if anything, goitrogenesis was more intense when triiodothyronine was co-administered.

One hour thyroid to plasma ratios of fetal and maternal I^{131} concentration (Fig. 4) were of interest in providing support of the gross and microscopic impression that the normal fetal thyroid is a relatively hyperplastic gland with a T/S ratio three times that of the adult.

B. *Goiter Production in Non-Pregnant Adult Female Guinea Pigs*

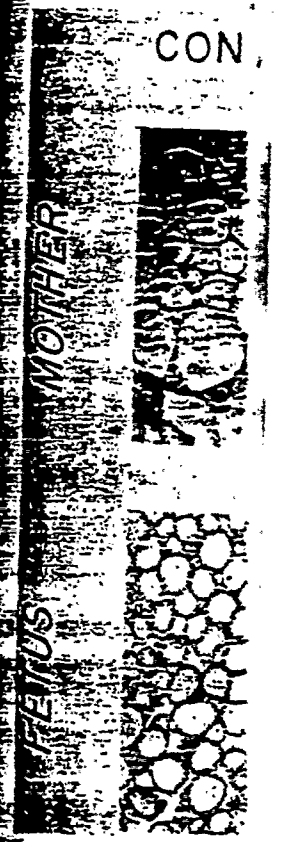
The absence of changes in maternal thyroids after treatment with $KClO_4$ raised the question as to whether or not it is possible, under any conditions,

FIG. 2. *In situ* appearance of thyroid glands of fetuses of 1 kg. mother who received 16 μg. triiodothyronine per day in addition to 1% $KClO_4$. Excised normal sized maternal thryoid is shown above.

to produce perchlorate goiter in adult guinea pigs. Furthermore, the possibility that pregnancy, as such, protects the maternal thyroid had to be considered. One per cent $KClO_4$ solution was therefore fed to non-pregnant adult female guinea pigs. Thyroid weights, histology, "T/S" ratios and iodine content were determined in control and perchlorate treated animals at 30, 60, and 90 day intervals. As shown in Figure 5, there was no evidence, by weight or histology, of perchlorate effects at 30 days, despite definite iodine depletion from 44 ± 22 μg./100 gm. to 13 ± 3.9 μg./100 gm. At 60 days, however, thyroid enlargement and intense hyperplasia were obvious. Inasmuch as the mean duration of exposure of the pregnant sows to 1% $KClO_4$ was only 37 days (range 21–48), it is reasonable to assume that insufficient time had elapsed for goiter to develop in the pregnant pigs.

C. *Partition of I^{131} Labeled Triiodothyronine in Mother and Fetus*

The placental transfer of administered T-3 was tested directly by injection of I^{131} labeled triiodothyronine (T-3*) into pregnant guinea pigs near



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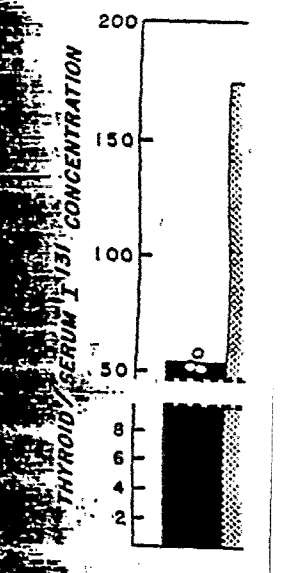

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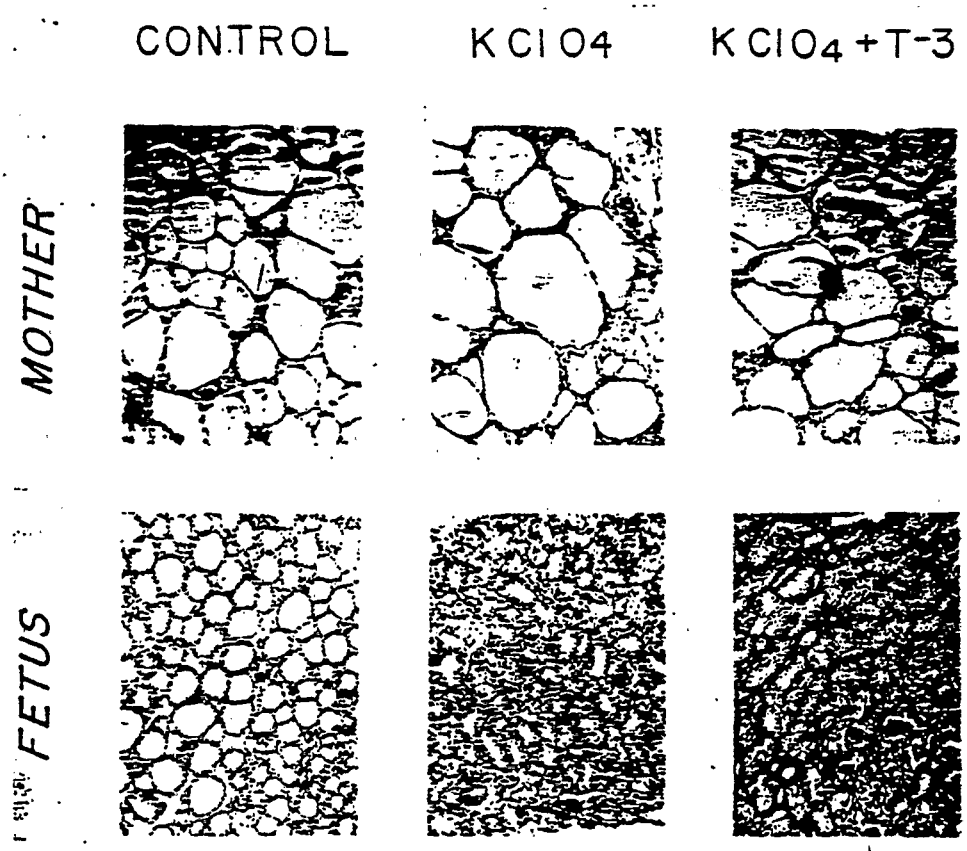

FIG. 3. Microphotographs of thyroid glands of adult pigs and their fetuses with and without daily administration of 32 μg. triiodothyronine.

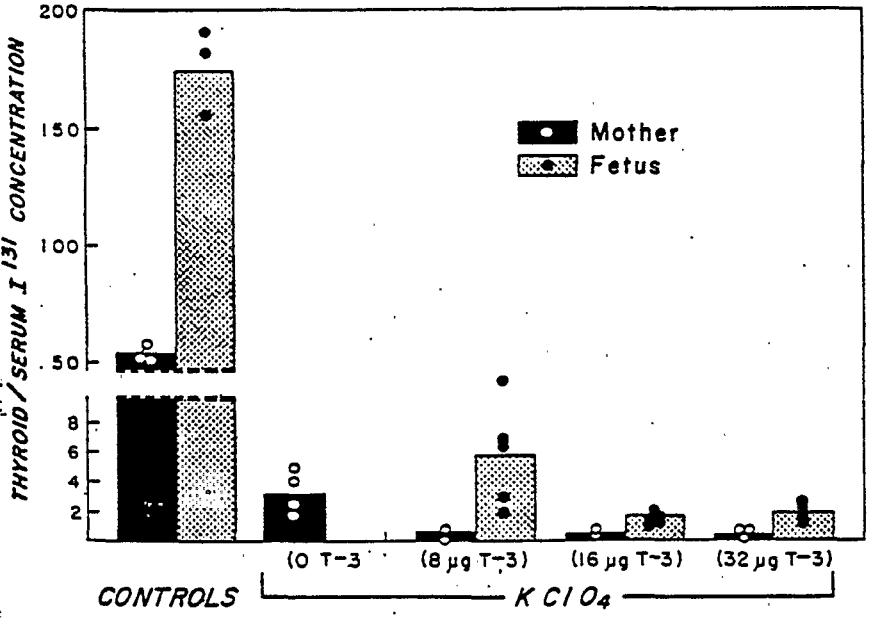

FIG. 4. One hour "T/S" ratios of fetuses (cross-hatched) and mothers (black bars) with and without graded doses of T-3. No blood was obtainable from fetuses of mothers receiving $KClO_4$ alone. Note break in scale.

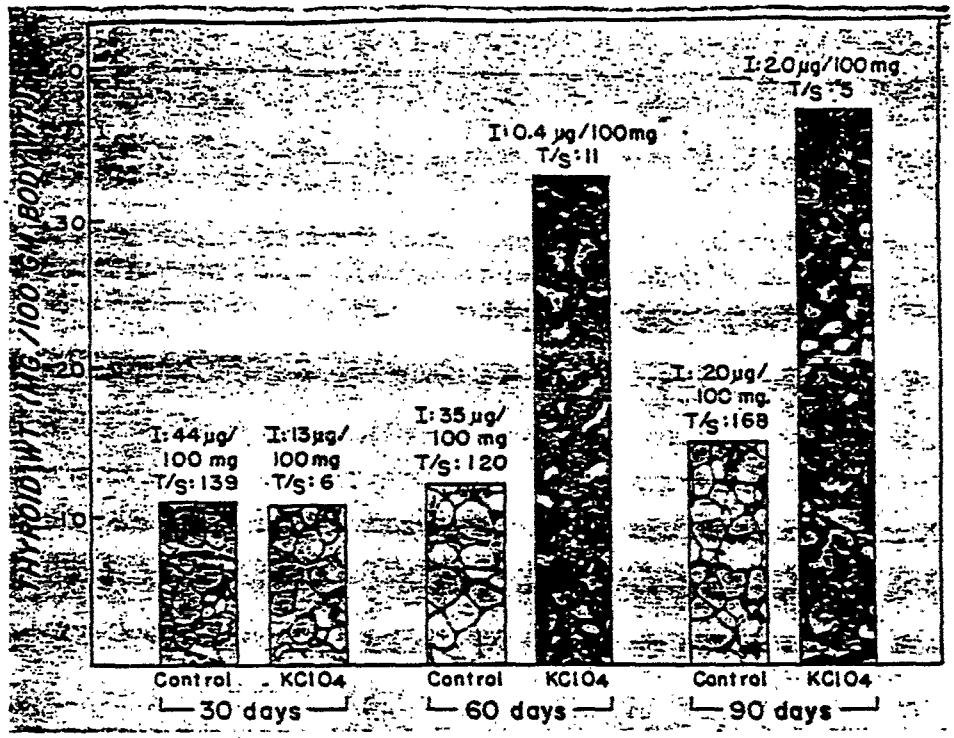

FIG. 5. Mean weights (mg./100 gm. body weight), iodine content, "T/S" ratios and representative histology of adult female guinea pig thyroids after 30, 60, and 90-day periods of treatment with 1% $KClO_4$. Four pigs in each of the six groups.

term. Concentrations of total radioactivity and T-3* in maternal and fetal plasma were determined at intervals up to 24 hours. Figure 6 shows the change in concentration of total radioactivity with time, the lower curve representing fetuses, the upper one the corresponding maternal concentration. In Figure 7 the *ratio* of fetal to maternal concentration is plotted against time. It is evident that the concentration of total I^{131} is not uniform in maternal and fetal plasma until 24 hours have elapsed. Furthermore, delay in equalizing total I^{131} concentration appears to be a function of the rate at which iodide becomes available by degradation of T-3* as indicated in the decay curves (Fig. 8) for chromatographically identified T-3* in the maternal and fetal circulations. These show that fetal and maternal vascular spaces equilibrate at a concentration ratio of 0.05 and that the biological half life of T-3* under these conditions is approximately 10 hours.

D. *The Mechanism of the Concentration Gradient for Labeled Triiodothyronine*

The above noted gradient for concentration of maternally administered T-3* across the placenta could be maintained by any one of the following mechanisms:

1) relative non-permeability of the placenta to T-3*,
2) rapid fetal degradation of freely diffused T-3*,
3) dilution of T-3* by a large quantity of stable T-3 in the fetus,
4) diversion of T-3* into extravascular depots,
5) exceeding the limit of total mass of T-3 which can be transferred across the placenta per unit time even if T-3 freely traverses it.

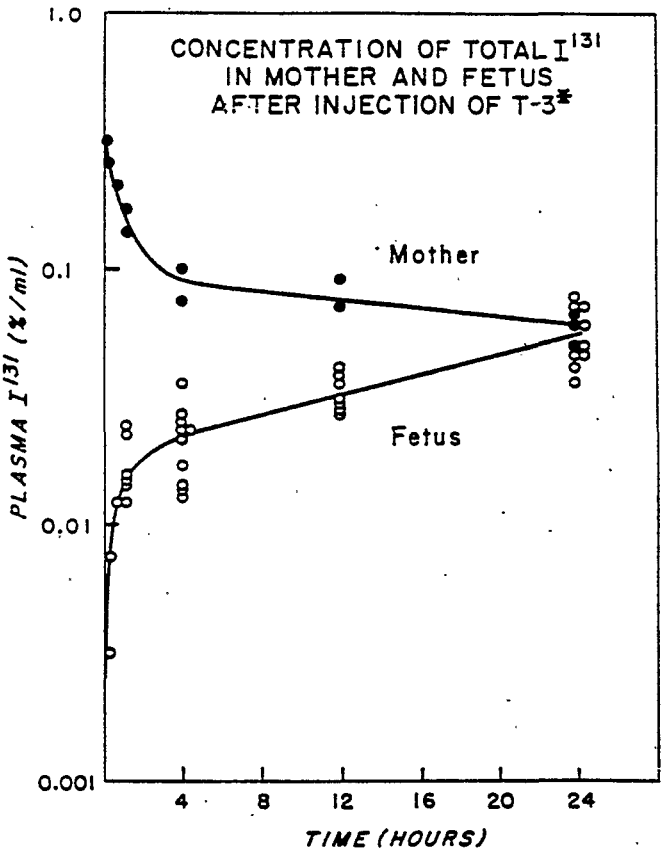

FIG. 6. Change in concentration of total radioactivity in maternal and fetal plasma after administration of I^{131} labeled triiodothyronine to pregnant guinea pigs.

Several experiments relative to consideration of these mechanisms were done. T-3* was administered to pregnant pigs intravenously together with 2.0 mg./kg. of carrier T-3 in one case and 20.0 mg./kg. in the other. The placental gradients for T-3* concentration at one hour were not significantly different (Table 1) from the gradients with microgram doses. This is in keeping with the observation that in all other experiments the fetal/maternal ratios did not correlate with variation in T-3 dose over a range of 0.3 to 10 μg.

The whole carcasses of 4 fetuses were homogenized and assayed for total

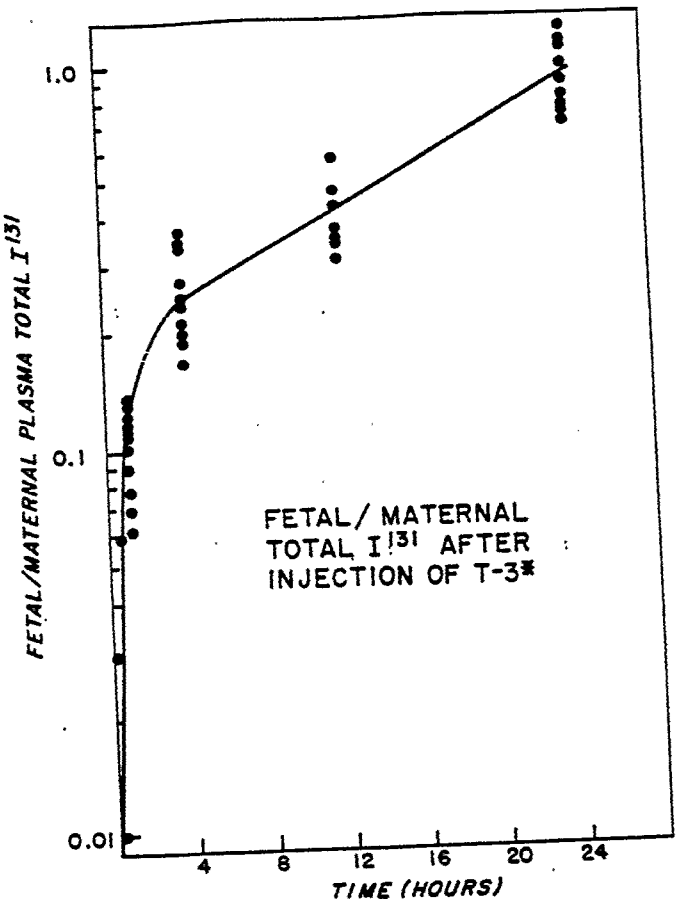

FIG. 7. Ratio of fetal to maternal plasma concentration of total radioactivity after administration of I^{131} labeled triiodothyronine to pregnant guinea pigs.

and T-3* radioactivity one hour after intravenous injection of T-3* into the pregnant sow. As recorded in Table 2, the concentration of T-3* in the body of the fetus was less than that in plasma, on a gram for gram basis

TABLE 1. INFLUENCE OF DOSE OF TRIIODOTHYRONINE ON DISTRIBUTION OF I^{131} LABELED TRIIODOTHYRONINE ONE HOUR AFTER INJECTION

	Dose of T-3 (mg./kg.)					
	0.005		2.0		20.0	
	Total I^{131}	T-3*	Total I^{131}	T-3*	Total I^{131}	T-3*
Ratio of Fetal to Maternal Plasma Concentration	0.13 0.13 0.10 0.11	0.026 0.030 0.023 0.022	0.13 0.16 0.14	0.035 0.072 0.044	0.104 0.113 0.102	0.018 0.032 0.027
Mean	0.12	0.025	0.14	0.050	0.11	0.026

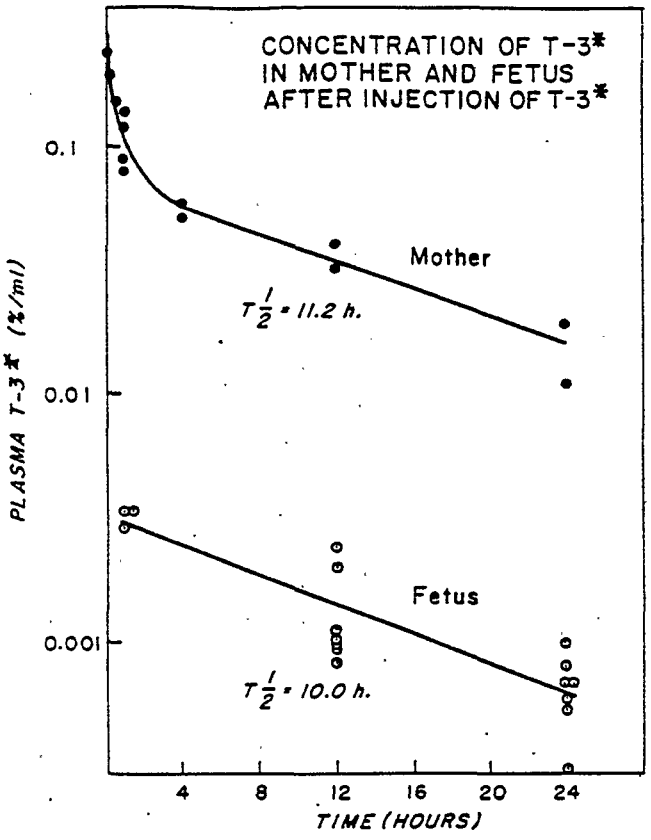

FIG. 8. Concentration of chromatographically identified triiodothyronine in maternal and fetal plasma after administration of I^{131} labeled triiodothyronine to pregnant pigs.

An independent estimate of the lifespan of T-3* in the circulation of the fetus was approximated by administering T-3* to newborn guinea pigs, i.e., 24 hours or less old. Decay of 1 and 10 μg. doses was, as can be seen from Figure 9, only slightly faster than for T-3* which traversed the placenta, i.e., $T_{1/2}$ 7.6 and 9.2 hours respectively, compared with 10.0 hours.

TABLE 2. BODY CONTENT OF TOTAL I^{131} AND T-3* ONE HOUR AFTER INJECTION OF T-3* INTO PREGNANT PIG

	% of total activity recovered	% of total activity/gm. body wt.	% of dose as T-3*/gm. body wt.	Ratio of total body to plasma T-3* concentration
Mother	93	.093	.076	0.38
Fetus 1	1.0	.018	.0027	0.61
Fetus 2	0.9	.018	.0017	0.47
Fetus 3	1.0	.016	.0020	0.91
Fetus 4	0.9	.014	.0009	0.50

This small difference presumably reflects the relatively minor contribution of placental transfer to the fetal disposal curve. The obvious differences in experimental conditions and the lack of specific activity data do not justify calculation of the rate constant for placental transfer on the basis of comparison of fetal and newborn decay curves. On the other hand, using T-3 degradation by the newborn pig as a reasonable gross index of events in the

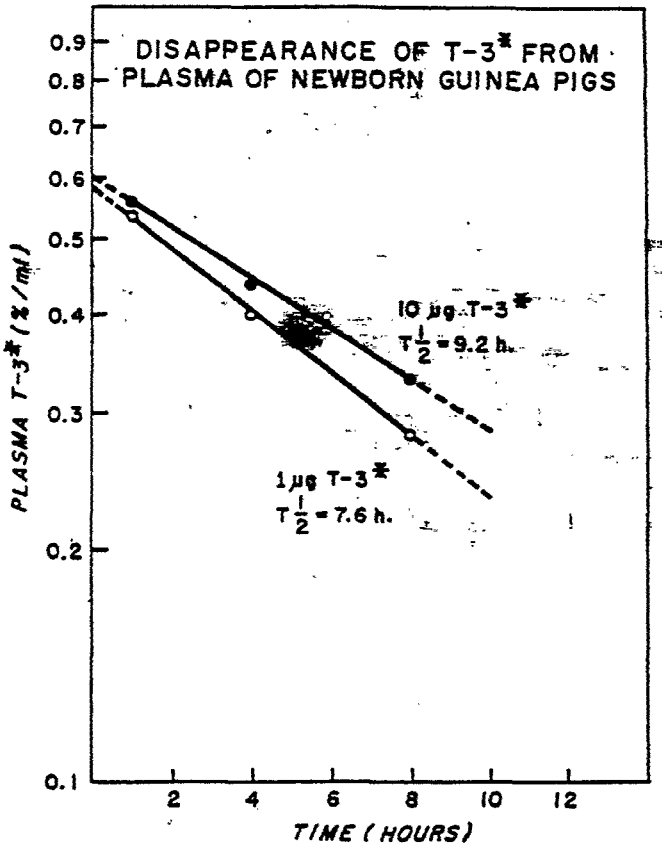

FIG. 9. Decay of labeled triiodothyronine in the plasma of newborn guinea pigs. Each point represents the mean value for four animals.

near term fetus, it would seem that fetal degradation is not the primary factor determining the observed fetal/maternal T-3* concentration gradient. The unlikelihood of this possibility is also indicated by the delay in equilibration with respect to total I^{131} (Fig. 7) and indirectly by failure of massive T-3 doses to modify the fetal/maternal ratios (Table 1).

Similarly, there is no evidence of a large fetal T-3 space to account for the low fetal T-3* concentrations. In the first place, it seems inconceivable that a 20 mg. dose of T-3 could distribute itself with a concentration

gradient of 0.05 by virtue of low specific activity on the fetal side. Secondly, the extrapolated volume of distribution of labeled T-3* (Fig. 8) is the same for the pregnant adult as for the newborns (Fig. 9). Finally and most directly, the total carcass analyses yielded concentrations lower than in plasma. This last observation also eliminates the possibility of pooling of T-3* in some extravascular fetal depot.

The very nature of the placenta as a massive surface for exchange rules out limited total penetration as a factor in the 0.05 fetal/maternal ratios. The results of the experiments with massive doses of carrier T-3 testify

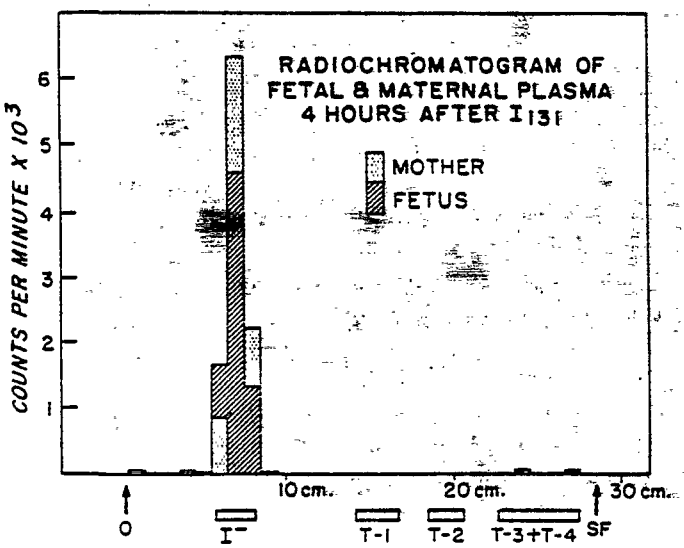

FIG. 10. Radiochromatogram of fetal and maternal plasma 4 hours after injection of I^{131} to perchlorate blocked pregnant pig.

directly to its efficiency in permitting maximal penetration of materials and eliminate this factor from further consideration.

It seems reasonable to conclude that the low concentration of T-3* in the fetus following its administration to the mother is due to relative non-diffusibility of the T-3 molecule or its protein carrier across the placenta. The small quantities of T-3* which *do* appear in fetal plasma are not due to exchange of I^{131} with I^{127} in preformed T-3 or to fetal incorporation of released I^{131} into its own hormone products despite pre-administration of $KClO_4$. These possibilities were ruled out by investigation of iodine compounds in the plasma of fetuses 4 hours after administration of I^{131} to the mother one-half hour after a 330 mg. dose of $KClO_4$. Figure 10 shows that only iodide is present in maternal and fetal plasma. In addition, there was no evidence of thyroxine in any maternal or fetal chromatograms after injection of T-3*.

COMMENTS

Production of massive thyroid enlargement in fetuses without effects on pregnant adult thyroids is in keeping with the concept of independence of the fetal pituitary-thyroid axis, as shown more directly by Nikitovich and Knobil (3) in the rat. It is reasonable to ascribe fetal goiters to entrance of perchlorate into the fetal circulation and not to maternal thyrotropin because there is no evidence of heightened thyrotropin activity in the mothers. The apparent extreme fetal sensitivity to iodine depletion by perchlorate reflects the intrinsic hyperactivity of the fetal thyroid as shown by its relatively large mass, comparative histology, and iodine concentrating ability. This apparent hyperfunction probably also applies to thyroid hormone production judging from the more rapid appearance (4) of radiothyroxine in fetal than in maternal thyroglobulin, and a conversion ratio which was higher in the fetal than in the maternal bovine circulation. It is not inconceivable that fetuses concentrate perchlorate and in doing so develop goiter more readily, but this possibility has not been investigated.

Administered T-3, on the other hand, is apparently effectively excluded from the fetus. This conclusion is based upon (a) the failure of large doses of T-3 to prevent or modify perchlorate induced fetal goiter and (b) the partition of labeled T-3* across the placenta with a fetal plasma concentration only one-twentieth that in the maternal circulation. Rapid degradation of T-3, though not responsible for the observed placental gradient, places further limitations on the concentration which can be achieved and maintained in the fetus for 24 hours following a single dose.

These observations are in keeping with the fate of labeled thyroxine administered to pregnant rabbits (5)[5] in that the fetal/maternal plasma ratio of labeled thyroxine, identified as the butanol soluble fraction, exceeded 0.054 in only one of the 23 rabbits. At four hours, practically all of the fetal radioactivity was present in the butanol soluble fraction, i.e., presumably as thyroxine. Barring species differences, this suggests that the major difference between administered thyroxine and T-3 from the point of view of access to the fetus is the more rapid degradation of the latter. This conforms to the known differences between T-3 and thyroxine turnover rates in rodents (6, 7) and implies that a dose of thyroxine, though equilibrated across the placenta in much the same fashion as T-3, survives longer and thereby exerts more pharmacologic activity. This difference probably accounts for the *partial* suppression of propylthiouracil induced fetal goiter in guinea pigs (8) when the mother received 25 μg. of dl-thyroxine daily. The same applies to the findings of Theresa (9) who noted microscopic involutional changes in fetal thyroids when pregnant rabbits

[5] The author is grateful to Drs. Hall and Myant for providing the opportunity to see their manuscript prior to its publication.

were given 0.05–0.1 gm. of thyroid powder. Reconsidering the data of Peterson and Young (8) and Theresa (9) in relation to the present study and the experiments of Hall and Myant (5), the weight of present evidence supports the original contention of Courrier and Aron (10) that administered thyroid hormones do not readily enter the fetal circulations of experimental animals. That this may also be the case in man is suggested by the report of goiter in the infant of a woman who received 500 μg. of thyroxine daily while taking propylthiouracil (11). The apparent similarities between the partition of thyroxine and triiodothyronine at the placental interface run somewhat counter to the concept of greater penetrability of trriodothyronine (6) and merits more direct comparative study.

Finally, the failure to observe a concentration gradient for iodide across the placenta in the present study suggests that perchlorate, like thiocyanate (1), interferes with its development.

SUMMARY AND CONCLUSIONS

Administration of $KClO_4$ to guinea pigs during the latter half of pregnancy resulted in large goiters in fetuses, but did not produce maternal goiter. In non-pregnant females thyroid enlargement occurred after 60 days of $KClO_4$ ingestion but not after 30 days, although some iodine depletion occurred over the shorter interval. Perchlorate-induced fetal goiter was not modified by simultaneous parenteral administration of *l*-triiodothyronine (T-3) in graded doses up to 32 μg. per day.

During the 24 hours after its administration, the concentration of I^{131} labeled T-3* in fetal plasma was only one-twentieth of the maternal concentration. Over this interval there was parallel decline in fetal and maternal T-3* concentration, corresponding to a biological half life of 10–11 hours. The ratio of the concentration of total I^{131} in plasma of fetuses to that of the mothers approached unity at 24 hours. Introduction of as much as 20 mg. of carrier did not modify the concentration gradient for T-3*, and there was no evidence of a large T-3 space to account for the observed discrepancy between maternal and fetal plasma concentrations of labeled T-3*. The biological half life of labeled T-3* in the plasma of newborn guinea pigs was of the same order as that of T-3* derived from the maternal circulation indicating that extremely rapid fetal degradation is not responsible for the fetal, maternal concentration gradient.

It is concluded that:

1) Perchlorate ion crosses the placenta and is a potent goitrogen in the guinea pig fetus by virtue of the sensitivity of the intrinsically hyperplastic fetal thyroid to iodine depletion.

2) Triiodothyronine administered to the pregnant guinea pig is ineffective in fetal goiter prevention because the placenta restricts its entry into the fetus, and it is rapidly degraded.

Acknowledgments

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